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Comm.
Dr. Gardner
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Dr. Sommers
Dr. Wyatt

PULMONARY

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8885

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Application for Research Grant
(Use extra pages as needed)

Date: July 30, 1973

1. Principal Investigator (give title and degrees):
Carroll E. Cross, M.D., Associate Professor and Director, Section of Pulmonary Medicine
Co-investigators: Elliot Goldstein, M.D., Assoc. Prof., Section of Infectious Diseases
Ching Chow, Ph.D., Assistant Research Biochemist, U.C. Davis Pulmonary Research Lab.
Mohammad Mustafa, Ph.D., Assistant Research Biochemist, U.C. Davis Pulmonary Res. Lab.

2. Institution & address:

Regents of the University of California
University of California at Davis
Davis, California 95616

3. Department(s) where research will be done or collaboration provided:
Department of Internal Medicine (Drs. Cross and Goldstein)
Department of Biological Chemistry (Dr. Mustafa)
Primate Center Research Facility (Drs. Mustafa and Chow)

4. Short title of study:
Cigarette Smoke Effects on Certain Aspects of Rat Lung Metabolism

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 30 months

7. Brief description of specific research aims:

The proposed studies are intended to accomplish the following specific research aims:

i) to determine the effects of long-term cigarette smoking on rat lung enzymes related to the pentose shunt and to glutathione metabolism; on rat lung lysosomal enzymes; on rat lung alveolar macrophages; and on rat anti-bacterial defense mechanisms.

ii) to determine the effects of long-term cigarette smoking minus particulates on the above mentioned lung biochemical and antimicrobial parameters

iii) to determine the interacting effects of long-term cigarette smoking and oxidant pollutant (ozone) exposure on the above mentioned lung biochemical and antimicrobial parameters.

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8. Brief statement of working hypothesis:

Preliminary studies in our laboratory have demonstrated that experimental rat exposures to cigarette smoke four times daily for five weeks result in significant increases in lung glucose-6-phosphate dehydrogenase (a key enzyme in the pentose shunt that subserves generation of "reducing equivalents") and increases the number of resident alveolar macrophages. It is thus our working hypothesis that long-term cigarette smoking does effect lung parenchymal metabolic processes, that these effects result in augmentation of certain enzymes related to pentose shunt activity, and that this could conceivably result in an augmented lung antioxidant defense capability. We intend to test this hypothesis by determining changes induced by cigarette smoking on the activities of a number of enzymes subserving lung glutathione metabolism and the superimposed effects of cigarette smoking and oxidants on these systems. We intend to determine if the particulate phase contributes to this metabolic adaptation by looking at the effects of both whole cigarette smoke and cigarette smoke without particulates on the forementioned lung metabolic parameters.

Since increases in resident alveolar macrophages have been reported to occur in human smokers we also propose to look at the number of alveolar macrophages that can be obtained by alveolar lavage in cigarette smoking rats and attempt to correlate this with particulate inhalation and with a simultaneous assessment of lung antibacterial defense systems.

Since we have found that high dose short-term oxidant exposures compromise lung antioxidant defense systems whereas low dose long-term oxidant exposures augment lung antioxidant defense systems, we plan to examine the effects of prior cigarette smoke exposure on lung short-term and long-term oxidant susceptibility.

Finally, since cigarette smoking causes an increase in lung alveolar macrophages in humans and since these cells have a high content of potentially damaging lysosomal enzymes, we plan to assess potential cigarette-induced lability (or intrapulmonary release) of these lysosomal enzymes by assessing both particulate phase and cytosol phase lysosomal enzyme activity in lungs exposed to cigarette smoke.

9. Details of experimental design and procedures (append extra pages as necessary)

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I. Introduction

The lung is an organ at risk from the effects of noxious inhaled environmental agents. As such, the lung has been provided with a number of self-protective defense systems. This study is designed to study the effects of cigarette smoking on two of these defense systems, mainly lung antioxidant defenses and lung antibacterial defenses. In addition, the study will include assesssments of the interacting effects of cigarette smoking and exposure to oxidant pollutants and assessments of lung lysosomal enzyme systems.

It has been shown recently that sulfhydryl-containing compounds are susceptible to such oxidant stresses as ionizing radiation (1), high oxygen tension (2), and ozone (3-5). These compounds, which are primarily glutathione and cysteine, have been postulated to be part of an antioxidant defense system in lung cells (6). Preliminary studies in this laboratory have suggested that although chronic cigarette exposure does not particularly affect the total amount of sulfhydryl-containing compounds in the lung, this exposure does alter the ratio of reduced to oxidized compounds in the cell (see III B). This alteration has a potential effect on the ability of the lung to withstand oxidant stress. For this reason, this study proposes to systematically characterize cigarette smoke-induced changes in lung glutathione and cysteine concentrations. This characterization will include chromatographic analysis of sulfur-containing compounds and spectrophotometric analysis of these compounds.

Previous reports have suggested that pretreatment with certain compounds give a measure of protection from subsequent oxidant challenge. For example, alpha-naphthylthiourea induces tolerance to high oxygen tension (8). Sublethal doses of ozone protect an animal from challenging doses of ozone (9). Considering the fact that cigarette smoke contains measurable amounts of over 250 different compounds, including a number that can be considered oxidants (10), it is possible that preexposure of animals to cigarette smoke can affect the animals subsequent susceptibility to oxidant stress.

Chow and Tappel have proposed an enzymatic mechanism for disposing of lipid peroxides generated by oxidants (11). There exists some data to support this mechanism (12,13). Cigarette smoke has been shown to alter certain enzyme activities. Most notably studied has been benzpyrene hydroxylase (14-17). Although there is considerable data that whereas the effects of whole cigarette smoke and volatile phase only (particulates removed) have been elucidated with regard to the ciliostatic effects of cigarette smoke and with regards to the pulmonary alveolar macrophage antibacterial defense system of the lung, there has been little study of the differential effects of whole smoke as compared to volatile phase on lung tissue biochemical responses to cigarette smoke.

Our preliminary data shows a cigarette smoke-induced augmentation of the activity of glucose-6-phosphate dehydrogenase in lung tissue (see III C). This suggests that cigarette smoke could potentially augment other enzymes related to the pentose cycle and to antioxidant defense systems. In the current proposal we plan to study further this aspect of cigarette smoke effects on the lung and to elucidate further the part played by particulates and by volatile phase on this response.

Recent studies performed by a colleague of ours at U.C. Davis have shown that ozone exposure increases lysosomal enzyme activities (18). Observations from other laboratories have indicated that cigarette smoke exposure increases lysosomal enzyme activities in pulmonary macrophages (19). Since increased activities of proteolytic lysosomal enzymes have been implicated in the

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pathogenesis of pulmonary diseases (20), especially in hereditary alpha$_1$-antitrypsin deficiency (21,22), it is important to establish whether there is any additive effect of combined cigarette smoke exposure and ozone exposure on lung lysosomal enzyme activities. This project intends to study the interaction of cigarette smoke exposure and ozone exposure as seen in lung lysosomal enzyme systems.

One of the thrusts of the current proposal is to study the combined interacting effects of cigarette smoke exposure and ozone exposure on aspects of lung pathophysiology. This is apparent from comments made above concerning the combined effects of cigarette smoke and ozone exposure on lung antioxidant defense systems and on lung lysosomal enzyme systems. Two further examples can perhaps be mentioned here.

It is known that ozone exposure produces pulmonary edema, with the extent of pulmonary edema determined by dose (23,24). Cigarette smoking is not known to induce pulmonary edema. However, exposure to cigarette smoke might inhibit or potentiate ozone-induced pulmonary edema. This project intends to study ozone-induced pulmonary edema and any effect that cigarette smoke might have on this edema. Of particular interest is the very low-grade edema associated with relatively low doses of ozone. Since these relatively low doses of ozone are found in the atmosphere of many major cities, it is of interest to examine the interacting effects of cigarette smoke exposure and low-dose ozone exposure on low-grade pulmonary edema. This edema can be sensitively measured by the method of Alpert et al (25), involving the measurement of lung accumulation of radioactively labelled protein.

Finally, it has been shown that ozone exposure decreases pulmonary bacterial clearance (26). Furthermore, cigarette smoke exposure is known to decrease pulmonary bacterial clearance (27). It is of interest to determine whether there are any additive effects of cigarette smoke exposure and ozone exposure on pulmonary bacterial clearance.

II. Methods and Procedure

Subjects will be 30-day old male Sprague-Dawley rats which are free of chronic respiratory diseases. In general, the 30 day old rats (or alternatively 60 day old rats) will be exposed to cigarette smoke for 5-10 week long periods after which they will be subsequently or concommitently exposed to room air or oxidant pollutants. At the conclusion of a predetermined exposure period a variety of studies concerned with lung metabolism will be done.

A. Cigarette exposures. These will be carried out in a Walton Horizontal Cigarette Smoking Machine (or alternative model). Initial studies will be accomplished on the combined volatile and particulate phase of cigarette smoke whereas a second series of studies will involve biological assessment of the volatile phase only, the particulate phase being filtered out by the Cambridge Filter. Exposures will be for 4 times daily, 10 minutes each, for 5-10 weeks utilizing the IRI 1A3 alkaloid series reference cigarette under standardized conditions of flow, volume and temperature.

For rat exposures the rats will be divided up into groups similar to groupings shown in III B. Some of the groups will, subsequent to cigarette smoke exposure, receive exposure to oxidant pollutants.

B. Ozone exposures. Ozone exposures will be carried out in specially designed chambers at the U.C.D. Air Pollution Exposure Facility. Ozone is

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generated by passing oxygen thru an electric silent arc ozone generator (28). The ozone is conveyed to the chambers at a flow of 100 liters per minute. The closed-system chambers have a volume of (27 cu ft). Air flow through the chambers is such that there are 10 changes of air per minute. These chambers are equipped for any range of ozone exposure from 0·1 ppm to 10 ppm. The temperature is maintained at 75° and the relative humidity is maintained at 50% .

C. Antibacterial defenses. Pulmonary bacterial clearance will be measured according to the method described in Goldstein et al. (29). Briefly, this method consists of offering the animals an aerosol of radiolabeled micro-organisms. After a suitable incubation period, the animals are sacrificed and the lungs are homogenized. The homogenate is assayed for the radiolabel, which is a function of the number of bacterial cell bodies remaining in the lungs. The lung homogenate is also cultured for visible bacteria, to assess pulmonary bacterial clearance. These two determinations give an indication of the number of organisms cleared from the lung per unit time, and also the number of organisms killed per unit time. This method produces an assessment of the extent of pulmonary anti-bacterial defense mechanisms.

D. Enzyme determinations. At the conclusion of the exposures rats will be weighed, anesthetized with pentobarbital and decapitated. Each set of lungs will be perfused with cold saline, and homogenized in isotonic sucrose-mannitol-Tris buffer with a glass-Teflon homogenizer. The homogenate will be filtered through cheesecloth and brought to 13 ml total volume by addition of buffer. The homogenate will then be apportioned for the particular assays to be done.

Glucose-6-phosphate dehydrogenase will be assayed according to the method of Lohr and Waller (30). Glutathione reductase will be assayed according to the method of Horn (31). Glutathione peroxidase will be measured according to the method described by Little and O'Brien (32). Protein content will be measured by the Lowrey method (33). In some experiments we will also determine overall comparative lung protein, RNA and DNA synthesis rates as determined by standard isotope incorporation rates.

Four specific proteolytic lysosomal enzymes will be studied. The peptidases cathepsin A, B, and C and the protease cathepsin D will be measured. The assay will be performed according to the method described by Misaka and Tappel (34). Three additional lysosomal enzymes will be studied. Lysozyme, acid phosphatase, and β-N-acetyl-glucosaminidase all appear to have relatively high specific activity in lung tissue (35). Lysozyme will be measured by a standard microbial bioassay technique (36). Acid phosphatase will be measured by the method of DeDuve et al (37). β-N-acetyl-glucosaminidase will be assayed according to the method of Beck (38).

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E. Morphological correlations. It is possible that exposure to cigarette smoke would induce a hyperplasia of the bronchial lining membranes and an increase in free macrophages in the regions of the terminal bronchioles and alveoli, as has been described in rodents following exposure to smog (39). As this could influence the biochemical observations being made, and in order to interpret the biochemical observations being made, it will be necessary to have morphological studies done in conjunction with the above outlined studies. Representative lungs from all the rat groups will thus be obtained for light microscopic and E.M. study under the direction of our University of California colleagues,

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Drs. Walter Tyler and Donald Dungworth of the U.C. Davis School of Veterinary Medicine. They are already doing extensive rat histological and E.M. studies as a part of our combined ozone exposure effects on rodent lung studies (40,41). We plan to attempt to correlate these anatomical sections with the number of free macrophages obtained by lung lavage, with lung DNA and protein synthesis rates as determined by isotope incorporation techniques and with the enzyme measurements discussed in D above.

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III. Prior Related Work

A. Publications Supported by the Council for Tobacco Research, U.S.A., 1970-73

C.E. Cross, U.C. Davis School of Medicine, Principal Investigator

1. Mustafa, M.G., and Cross, C.E.: Localization of Na+-K+, Mg++ ATPase in alveolar macrophage subcellular fractions. Life Sci., 9: 947-54, 1970.

2. Mustafa, M.G., Cross, C.E., Tyler, W.S., and Duffy, M.J.: Interference of cadmium ion with oxidative metabolism of alveolar macrophages. Arch. Int. Med. 127: 1069-77, 1971.

3. Brunstetter, M.A., Hardie, J.A., Schiff, R., Lewis, J.P., and Cross, C.E.: The origin of pulmonary alveolar macrophages. Arch. Int. Med. 127: 1064-68, 1971.

4. Cross, C.E., Mustafa, M.G., Peterson, P., and Hardie, J.A.: Pulmonary alveolar macrophage: Membrane associated Na+, K+, Mg++-Adenosine triphosphatase system. Arch. Int. Med. 127: 1069-77, 1971.

5. Mustafa, M.G., Peterson, P.A., Munn, R.J., and Cross, C.E.: Effects of Cd++ on metabolism of lung cells. Proceedings of the Second International Clear Air Congress, Edited by H.M. Englund and W.T. Berry, Academic Press, N.Y., p. 143-151, 1971.

6. Cross, C.E., Ibrahim, A.B., Ahmed, M., and Mustafa, M.G.: Effects of Cd++ on respiration and ATPase activity of the pulmonary alveolar macrophage: A model for the study of environmental interference with pulmonary cell function. Environ. Res. 3: 512-20, 1970.

7. Mustafa, M.G., Cross, C.E., Munn, R.J., and Hardie, J.A.: Effects of divalent metal ions on alveolar macrophage membrane adenosine triphosphatase activity. J. Lab. Clin. Med. 76: 563-71, 1971.

8. Cross, C.E., Le, C.T., and Mustafa, M.G.: Effects of ozone on some pulmonary alveolar macrophage (PAM) biochemical reactions. Clin. Res. 19:147, 1971 (abstract).

9. Mustafa, M.G., Cronin, S.R., Duvall, T.R., and Cross, C.E.: Ozone effect on activities of lung cellular membrane enzymes. Clin. Res. 20: 197, 1972 (abstract).

10. DeLucia, A.J., Hoque, P.M., Cross, C.E., and Mustafa, M.G.: Sulfhydryl content of rodent lungs under oxidant stress. Clin. Res. 20: 240, 1972.

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11. DeLucia, A.J., Hoque, P.M., Mustafa, M.G., and Cross, C.E.: Ozone interaction with rodent lung: Effect on sulfhydryls and sulfhydryl-containing enzyme activities. J. Lab. Clin. Med. 80: 559-66, 1972.

12. DeLucia, A.J., Mustafa, M.G., and Cross, C.E.: Glutathione and sulfhydryl-containing enzyme activities in lung under ozone stress. Clin. Res. 21: 223, 1973 (abstract).

13. York, G.K., Arth, C., Stumbo, J.A., Cross, C.E., and Mustafa, M.G.: Effect of cigarette smoke and tobacco extract on lung macrophage function. Arch. Environ. Health, in press, 1973.

14. Mustafa, M.G., DeLucia, A.J., York, G.K., Arth, C., and Cross, C.E.: Ozone interaction with rodent lung: Effects on oxygen consumption of mitochondria. J. Lab. Clin. Med., in press, 1973.

15. Mustafa, M.G., DeLucia, A.J., Cross, C.E., York, G.K., and Dungworth, D.C.: Effect of ozone exposure on lung mitochondrial oxidative metabolism. Chest, in press, 1974.

16. York, G.K., Cross, C.E., Mustafa, M.G., and Bodai, B.I.: Lung glucose-6-phosphate dehydrogenase stimulation by cigarette smoke. Effects of short-term ozone exposure. Submitted for publication, 1973.

17. Mustafa, M.G., and Cross, C.E.: Effects of short-term ozone exposure on lung mitochondrial oxidative and energy metabolism. Submitted for publication, 1973.

18. Mustafa, M.G., and Cross, C.E.: Lung cell mitochondria: Rapid oxidation of glycerol-1-phosphate but slow oxidation of 3-hydroxybutyrate. Submitted for publication, 1973.

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B. Preliminary Protocol January-June 1973,...A study of Cigarette smoke effects on selected aspects of lung metabolism.

i) Aim: To accomplish a preliminary study of the effects of cigarette smoke and cigarette smoke plus oxidants on selected aspects of lung metabolism.

ii) Introduction: It has been shown experimentally that chronic cigarette smoking influences lung biochemistry both in vivo and in vitro (42-48). It has also been shown that oxidant pollutants interact with a number of important lung biochemical reactions (49-51) including lung antibacterial defense systems (52). This protocol is intended to gather preliminary data on the effects of cigarette smoking and on the effects of cigarette smoking plus oxidant pollutant exposure on a number of lung enzyme systems known to be influenced by both high dose short-term and low dose long-term exposures to ozone. Since certain constituents of cigarette smoking represent oxidants (53) and since both cigarette smoking (43,44) and oxidant pollutants (51) are known to induce certain lung enzymes, this would seem a priori a reasonable hypothesis to test. One interesting question is whether cigarette smoke is capable of inducing enzymes which are proposed to be responsible for oxidant protection, i.e. glutathione reductase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase (51).

Another interesting question relates to the combined effects of cigarette smoke and oxidants on the "redox state" of lung tissue. Parameters suggested for study include lung glutathione levels and ratios of reduced to oxidized reactive lung thiol groups. Of course, it will be necessary to make concomitant histological observations in order to interpret the biochemical data being generated.

iii) Methods:

a. Cigarette smoke exposures

Thirty two male Sprague-Dawley CRD-free rats, aged 30 days, are the subjects of the experiment. These rats are to be weighed and divided into four groups of eight rats, as follows:

Group	Exposure Conditions
A	sham cigarette exposure, sham O_3 exposure (control group)
B	sham cigarette exposure O_3 3.0 ppm x 4 hr.
C	cigarette exposure 4/day x 35 days, sham O_3 exposure
D	cigarette exposure 4/day x 35 days, O_3 3.0 ppm x 4 hr.

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Half of the rats are to be exposed to cigarette smoke 4 times per day for 35 consecutive days. Each exposure consists of 9 puffs of a 85 mm non-filtered American cigarette. Reference IRI 1A3 cigarettes are to be used in the future. Each puff consists of 30 ml of smoke in a 378 ml chamber (i.e. 1:12 dilution). A Walton Horizontal Cigarette Exposure Machine is to be used for the exposures. The sham-exposed rats are to be placed in the chambers and given 9 puffs of air in a ten-minute period four times daily.

b. Ozone exposures

After 35 consecutive days of smoke exposure, two groups (B and D) of the rats will be subjected to 3.0 ppm O_3 for four hours. Ozone is to be delivered under standard conditions of flow and temperature at the Air Pollution Exposure Laboratory at U.C.Davis.

c. Experimental

Immediately after the ozone exposure, the rats are weighed, anesthetized with pentobarbital and decapitated. Blood is collected for complete blood counts. Lungs are to be perfused with cold saline and homogenized in isotonic sucrose-mannitol-Tris buffer with a glass-Teflon homogenizer. The homogenate is to be filtered through cheesecloth and brought to 16 ml total volume by addition of isotonic sucrose-mannitol-Tris buffer.

The following determinations will be made on the obtained lung tissue:

Homogenate Assays

- Reduced glutathione
- Oxidized glutathione
- Mitochondrial respiration
- Total sulfhydryls
- Non-protein sulfhydryls
- Oxidized non-protein sulfhydryls
- DNA determination
- Protein determination

Mitochondrial Assays

- NADH - cytochrome c reductase
- Succinate-cytochrome c reductase

Microsomal Assay

- NADPH - cytochrome c reductase

Cytosol Assays

- glucose-6-phosphate dehydrogenase

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d. Critique of initial protocol

Several factors of interest were not evaluated in this preliminary experiment. First, no histology or electron microscopy studies were done. Second, the lungs were not evaluated for any changes in cell population; of

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particular interest would have been an estimation of the number of type II cells and/or alveolar macrophages in both the control and the cigarette exposure groups. Third, an increased number of blood assays could have been performed, including evaluation of hematopoiesis. Fourth, the anti-oxidant enzyme systems of glutathione reductase - glutathione peroxidase were not evaluated in the preliminary experiment. Fifth, no determination was made of lung lysosomal enzyme systems, both total and compartmentalized, in the preliminary protocol. All of these defects will be remedied in future protocols.

iv) Results of first preliminary protocol

a. Meaning of groups

A = sham ozone exposure; sham cigarette exposure (control)

B = ozone-exposed; sham cigarette exposure

C = sham ozone exposure; cigarette exposed

D = ozone exposed and cigarette exposed

b. G-6-PD

In the G-6-PD assay, there was a significant difference between smokers and non-smokers (see Fig. 1). Group B was insignificantly elevated over Group A, but Group C was elevated 54% of control, and Group D was 32% of control. There is also a drop of 22% between Group C and Group D, suggesting the influence of ozone superimposed on the cigarette smoke effect on this enzyme.

c. Non-protein sulfhydryls

In figure 2, we see the results of reduced and oxidized non-protein SH determinations. There is a 12% drop in total NPSH (i.e. reduced NPSH) between Group A and Group B. There is an even further drop of 21% in non-reduced NPSH from Group A to Group B. This is an expected drop since the ozonized Group B would be expected to lose some NPSH to higher oxidation status.

There is only a very slight drop between Group A and Group C; this could signify that chronic exposure to cigarette smoke does not appreciably reduce NPSH levels.

There is a 9% drop in reduced NPSH between Group D and Group A, and a 5% drop between Group D and Group C. There is a 7% rise in non-reduced NPSH between Group C and D.

d. Glutathione

The ratio of GSH/GSSG dropped 44% from Group A to Group B (see fig. 3). This represents a significant relative oxidation of glutathione after oxidation by ozone. This is an expected result of ozone exposure. There was a 30% rise in GSH/GSSG between Group A and Group C representing relative reduction by cigarette smoke. This was not expected, but it may help to explain certain other features of this experiment. There was little difference in GSH/GSSG between Group A and Group D; this perhaps signifies that there is oxidation by ozone, but that the extent of oxidation is less between A and B than between C and D.

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There was a 43% drop in GSH between Group A and Group B (see Fig. 4). This was expected. What was not expected was a 21% drop in GSH between Group A and Group C, and no drop at all between Group C and Group D. This perhaps means that there is some other NPSH which is responsible for the slight drop in NPSH between C and D.

e. White cell count

There was no significant difference in white cell count between Group A, Group B, and Group C, although there was some drop between A and B. There was a significant drop between Group A and Group D.

f. Other assays

The other assays done (i.e. reductases and respiration) yielded insignificant data. Platelet counts and hematocrits showed no difference between control and experimental groups.

v) Methods, second preliminary protocol

Exposure conditions will be identical to the first protocol. We will eliminate platelet counts and perhaps the reductase assays. We will add macrophage counts, alveolar lavage albumin assay, and GSH reductase - GSH peroxidase assay.

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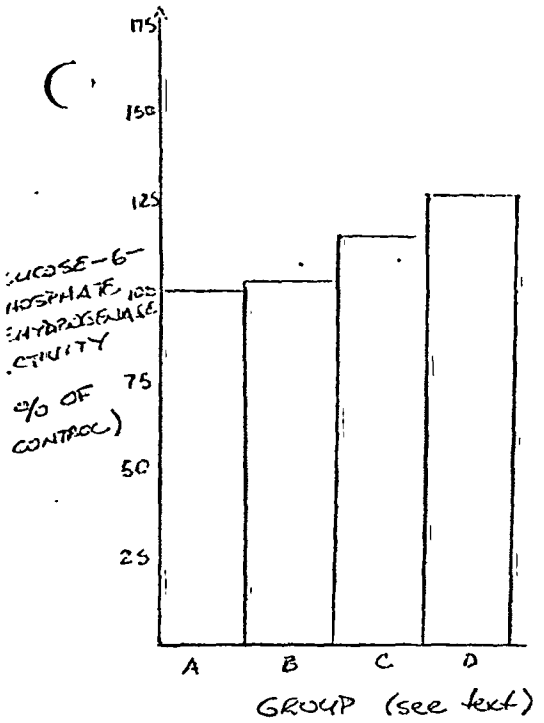

FIGURE 1.

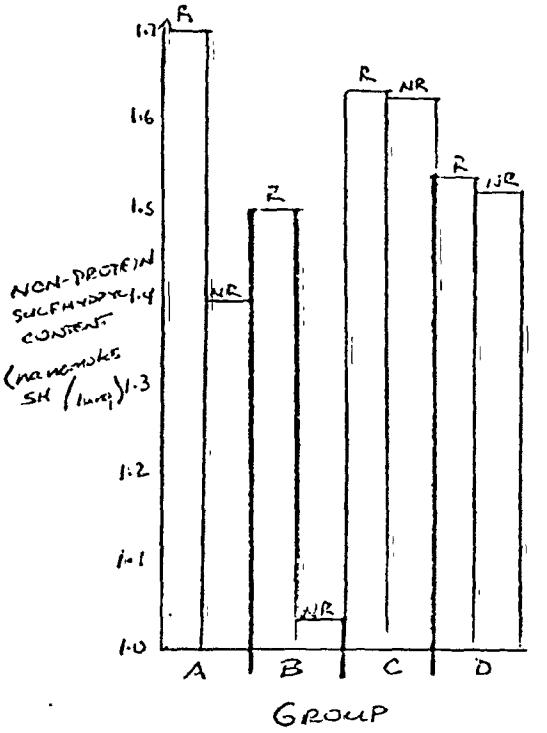

FIGURE 2.

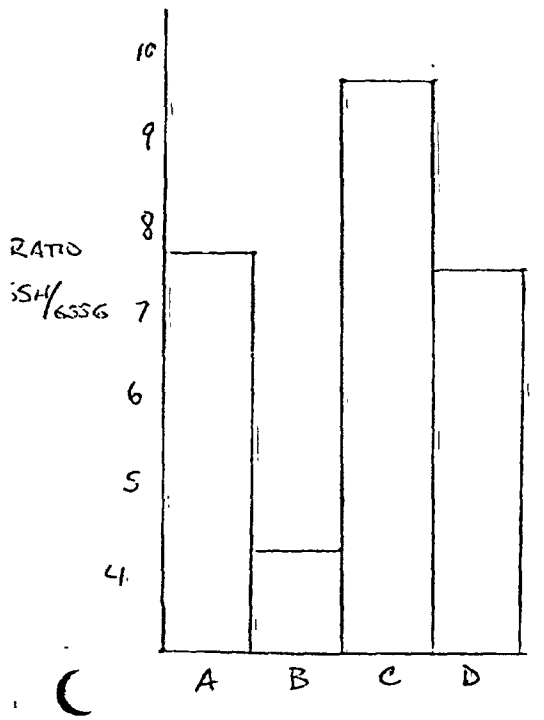

FIGURE 3.

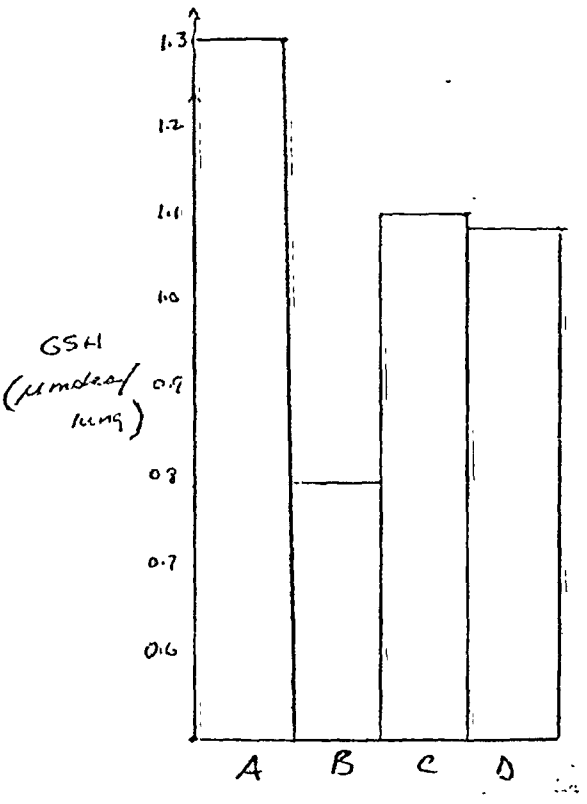

FIGURE 4.

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III. Preliminary Publication

STIMULATION OF LUNG GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY BY CIGARETTE SMOKE. EFFECTS OF SHORT-TERM OZONE EXPOSURE[1]

Recently three groups of investigators have demonstrated significant increases in glucose-6-phosphate dehydrogenase (G-6-PD) activities in lung tissue following oxidant exposure[2-5]. The significance of the augmented G-6-PD activity most probably relates to increased hexose monophosphate shunt activity required by reparative processes in the lung, e.g., increased nucleotide production (for nucleic acid synthesis) and increased NADPH production (required for a number of oxido-reductive biosyntheses)[6-8]. However, the augmented G-6-PD activity might also speculatively relate to an increased supply of NADPH available for the reduction of oxidized glutathione (GSSG), a mechanism operative in red cells and which may presumably relate to lung protective anti-oxidant defense capabilities.

Cigarette smoke exposure has been reported to modify a number of lung enzyme activities, including those related to alveolar macrophage function[9-11] and to lung metabolism[12-14]. This report describes the effects of cigarette smoke exposure on lung G-6-PD activity. Since both exposure to cigarette smoke and to air pollutant have been associated with bronchopulmonary diseases, including an increased susceptibility to respiratory infections[15,16], the effects of a superimposed acute O_3 exposure were also determined.

Forty 30-day-old Sprague-Dawley rats (originally specific pathogen free) were divided into four groups. Ten rats served as control and received 35 days of sham cigarette smoke exposure plus 4 hours of sham O_3 exposure; ten received 35 days of sham cigarette smoke exposure followed by 4 hours of exposure to 3 ppm O_3; ten received 35 days of cigarette smoke exposure followed by sham O_3 exposure; and ten received 35 days

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of cigarette smoke exposure followed by 4 hours of exposure to 3 ppm O_3.

The cigarette smoke exposures consisted of 9 puffs of cigarette smoke in a ten-minute period (Pall Mall 85 mm) 4 times daily for 5 weeks in a Walton Horizontal Cigarette Exposure Machine[17] set to give 1:12 smoke:air ratio at a puff volume of 30 ml. Sham cigarette smoke exposures consisted of puffs of room air delivered through the same apparatus. Ozone exposures consisted of 3.0 ppm (± 0.1) ozone for 4 hours in a modified fiberglass environment unit described previously[5,18]. Sham ozone exposures consisted of room air exposures in identical units.

Animals were anesthetized with pentobarbitol and exsanguinated. The lungs were perfused with cold isotonic saline via the pulmonary artery, then trimmed of excess bronchovascular and pleural connective tissue. The lungs were then homogenized with a glass Teflon homogenizer, using an isotonic medium (0·15 M sucrose, 0·15 M mannitol, 1 mM Tris). The homogenate was filtered through two layers of cheesecloth. The homogenate was then centrifuged at 600 x g for ten minutes to remove nuclei and cellular debris and subsequently at 37,000 x g for twenty minutes to remove mitochondria and microsomes. The supernatant (cytosol) was then used for enzyme assay.

G-6-PD activity in the cytosol was determined according to the method of Lohr and Waller[19]. Protein concentration was determined by the method of Lowry[20].

G-6-PD activities in lung cytosol from control, ozone exposed, cigarette exposed, and cigarette plus ozone exposed rats are shown in Table 1. Control rats showed an activity of 39.2 ± 9.1 nanomoles NADPH formed/min/mg protein at 23°C. Rats exposed to 3.0 ppm ozone for 4 hours showed a 12% decrease, similar to findings reported previously[5]. Rats exposed to cigarette

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smoke for 5 weeks showed a 28% increase relative to untreated control rats. In cigarette smoking rats subsequently exposed to ozone there was 7% increase over control rats but a decrease of 20% compared to the cigarette smoking group not exposed to ozone. Rats exposed to cigarette smoke and subsequently exposed to ozone showed a 21% increase over rats exposed to ozone only.

Lung G-6-PD activity has been the object of study by a number of investigators concerned with the injury-reparative processes in the lung, including that due to high tensions of oxygen[2], short-term ozone exposure[5], long-term ozone exposure[3,4] and silica dust inhalation[21]. Although G-6-PD activity represents only one enzymatic step in the hexose monophosphate shunt, it occupies a key position in overall glucose metabolism in the lung and has been used to estimate the activity of the hexose monophosphate pathway[22,23]. The observation that chronic cigarette smoking stimulates lung G-6-PD activity suggests that cigarette smoking augments overall activity of the hexose monophosphate shunt in lung tissue. The observation that short-term, high level exposures to ozone inhibits lung G-6-PD activity verifies previous data indicating that this oxidant inhalant under these conditions rather non-descriminantly decreases the activity of a number of lung enzyme systems[5,24].

The present studies do not allow further conclusions to be drawn concerning the mechanisms involved in the cigarette smoking induced increases in G-6-PD activity. It is possible that processes such as cellular hyperplasia, cellular proliferation or increased cell renewal could account for the augmented activity[6-8]. In addition, the question arises as to which cells in the lung are primarily affected by the smoke exposure. In this context it is particularly interesting that cigarette smoking exposure increases the number of alveolar macrophages appearing in the lung[25,26] and that these cells have been noted to possess an

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active pentose shunt metabolism[27]. In addition, it is an attractive assumption that the augmented shunt activity, by producing NADPH, might be involved in determining lung susceptibility to oxidant stress much as red cell G-6-PD activity and related NADPH generation help to determine red cell antioxidant defense capabilities[28]. This assumption will require further validation by determination of the activities of related anti-oxidant enzyme systems in the lung such as glutathione, glutathione reductase and glutathione peroxidase.

The composition of cigarette smoke is complex. Biological and biochemical testing of whole cigarette smoke should be conducted with this limitation in mind. The merit of the present biochemical assay of a parenchymal lung tissue response to whole cigarette smoke suggests that future studies with simpler extracts of cigarette smoke, such as with the particulate phase removed, would be a worthwhile undertaking.

In summary, the results demonstrate that experimental cigarette smoking in rodents for 35 days increases lung G-6-PD activity. Short-term exposures to high levels of ozone (3 ppm, 4 hrs.) decreases lung G-6-PD activity whereas combined cigarette smoke and short-term high level O_3 exposures give an intermediate effect. The neutralization of opposing effects of ozone and cigarette exposure, so that rodents exposed to both agents show little difference from control animals, suggests that there may be a diminished susceptibility of the smokers to oxidant exposure. The importance of this lung enzymatic effect with respect to overall lung function of the smoker is unknown.

George K. York[29]

Carroll E. Cross

Mohammad G. Mustafa

Balazs I. Bodai[29]

Section of Pulmonary Medicine, Departments of Internal Medicine and Biological Chemistry, School of Medicine, University of California, Davis, California 95616.

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Table 1. Effect of Long-Term Cigarette Smoke Exposure and Short-Term Ozone Exposure on Glucose-6-Phosphate Dehydrogenase Activity in Rat Lung Cytosol

Group	A	B	C	D
Exposure Conditions	Control	Ozone	Cig. Smoke	Cig. Smoke + O_3
No. of Rats	10	10	10	10
G-6-PD Activity (nanomoles NADPH formed/ min/mg protein)	39.19 ±9.1	34.79 ±12.8	50.16 ±16.3	42.10 ±18.6
% of Control	-	88%	127%	107%
P vs control (A)	-	<.2	<.05	n.s.
P vs ozone (B)	-	-	<.025	<.2
P vs cigarette (C)	-	-	-	<.2

Statistical analysis by Student's T test. n.s. = not significant

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IV. Significance

Although the effects of cigarette smoking on the population at large have been well documented (54), there is a paucity of information concerning possible mechanisms of cigarette smoke damage on the lung. This is especially true regarding interacting effects of cigarette smoke and lung antioxidant defense systems such as lung NADPH, glutathione and glutathione peroxidase systems and on the important potential interacting effects with lung lysosomal enzyme systems including those of the pulmonary alveolar macrophages. There is virtually no data concerning in vivo biological assessments of the contribution of vapor plus particulate phase components of cigarette smoke on aspects of lung metabolism vs effects of vapor phase only. Likewise, although there have been a number of studies suggesting important interactions between cigarette smoking and air pollution exposure, there is little firm data available that would scientifically establish whether or not these two agents would behave synergistically insofar as their effects on lung parenchymal biochemical response are concerned or insofar as lung antimicrobial responses are concerned.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The investigators have at their disposal four laboratory areas: i) the 500 square foot Pulmonary Disease Research Laboratory at the U.C. Davis School of Medicine. This laboratory is equipped to perform cigarette exposures and biochemical assays. ii) The 400 square foot Infectious Disease Research Laboratory at the U.C.D. School of Medicine. This laboratory is equipped to assess pulmonary antibacterial defense function. iii) Three adjacent 250 square foot laboratories at the U.C. Primate Research Center. These laboratories are equipped to perform tissue preparation, ultracentrifugation, and biochemical assays. iv) The 4,000 square foot Air Pollution Exposure Facility at the U.C. Davis Primate Research Center. This facility is equipped for all aspects of exposure of laboratory animals to air pollution.

11. Additional facilities required:

Should the proposed studies be approved by the Council it would be most desirable if we could obtain an additional Cigarette Smoke Exposure Apparatus for our animal exposures. This would be our highest priority. While the Walton Chamber is satisfactory, it is far from ideal. We would like to obtain, as a minimum, one of the modified revised Walton Chambers and most ideally we would like to obtain a chamber that would allow for more simultaneous exposures than can be presently accomplished (6 rats at a time).

There are no other major space or equipment needs to accomplish the proposed studies. The budget equipment items mentioned are all relatively minor. A table top centrifuge is requested to facilitate tissue processing. The present Walton apparatus requires certain modifications that are estimated to cost $2,000. Lastly, certain nebulizer modifications are required for assessment of the rat antibacterial defense capabilities.

12. Biographical sketches of investigator(s) and other professional personnel (append).

see attached c.v.s

13. Publications: (five most recent and pertinent of investigator(s), append list, and provide reprints if available).

see attached

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14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
* Carroll E. Cross, M.D.	10	
* Elliot Goldstein, M.D.	5	
Mohammed G. Mustafa, Ph.D.	40	
Ching K. Chow, Ph.D.	80	
George K. York, Ph.D. candidate in Path.	100	
Ph.D. candidate in Microbiology	25	
** 4 work-study students to work as cigarette exposure machine tech.	20	

REDACTED

Technical

None

* In keeping with University of California academic staff policy, 50% of research time is listed as salary

** Work-study program students are supported by $3 in Federal grants for each $1 in research funds.

Sub-Total for A REDACTED

B. Consumable supplies (by major categories)	
Biochemicals	$ 1,500
Radiophosphorus and radioiodine	1,000
Histochemical supplies	500
Microbiological supplies	1,000
Standard reference cigarettes @$10/1000	200

Sub-Total for B $ 4,200

C. Other expenses (itemize)	
1,000 rats @$5	$ 5,000
Medical illustration and xerox	500
Travel (2 meetings/year)	500

Sub-Total for C $ 6,000

Running Total of A + B + C $35,350

D. Permanent equipment (itemize)	
Table top centrifuge	1,000
Cigarette Chamber Modifications & additions	2,000
Nebulizer equipment (hand made, specially constructed for bacterial aerosol exposures)	1,000

Sub-Total for D $ 4,000

E 5,300

E. Indirect costs (15% of A+B+C)

Total request $44,650

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	REDACTED	4,000	6,000	2,000	5,400	43,400
Year 3		3,000	3,000	1,000	3,000	24,000

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16. Other sources of financial support.

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Pulmonary Effects of Environmental Oxidant Pollutants	U.S. Public Health Service (ES AP 00628-01)	$10,000*	June 1, 1971 – May 31, 1975

* This is a Program Project Grant for $310,866. Pollution chambers and some animal costs are covered by the Core support. My personal share is approximately $10,000/yr.

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Proposed Studies of the Effects of Oxidant Pollutants on Red Blood Cells and Lung Cells	California Air Resources Board	$40,300	October 1, 1973 – September 30, 1975

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Carroll E. Cross, M.D.

Signature Carroll E. Cross Date _____

Telephone R
Area Code Number Extension

Check payable to

_____

Mailing address for checks

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Responsible officer of institution

Typed Name _____

Title _____

Signature _____ Date _____

Telephone _____
Area Code Number Extension

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R: REDACTED MATERIAL

July 1, 1973

CURRICULUM VITAE

NAME — Carroll Edward Gross, M.D.

ADDRESS — Pulmonary Disease Research Laboratory
Department of Internal Medicine
School of Medicine
University of California
Davis, California 95616

DATE AND PLACE OF BIRTH — REDACTED

MARITAL STATUS — REDACTED

EDUCATION — Pomona College — REDACTED
University of Washington, School of Medicine,
Columbia University, College of Physicians and Surgeons -
REDACTED

MILITARY — U.S. Army National Guard, REDACTED

BOARD STATUS — Diplomate: American Board of Internal Medicine (1969)
Passed: Subspecialty Boards in Chest Medicine (1971)

POSITIONS (MEDICAL)

1961 - 1962	Intern, Mixed Medical, University of Wisconsin Hospital Center, Madison, Wisconsin
1962 - 1964	Residency, Internal Medicine, Stanford University, School of Medicine, Palo Alto, California
1964 - 1967	Fellowship in Pulmonary Diseases, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
1967 - 1968	Instructor of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
1968 - 1973	Assistant Professor of Medicine, University of California at Davis, School of Medicine, Davis, California
1969 - 1973	Assistant Professor of Human Physiology, University of California at Davis, School of Medicine, Davis, California
1973 - present	Associate Professor of Human Physiology and Medicine, University of California at Davis, School of Medicine Davis, California.

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23. Mustafa, M.G. and Cross, C.E.: The pulmonary alveolar macrophage: Oxidative metabolism of isolated cells and mitochondria and effect of cadmium ion on electron and energy transfer reactions. Biochemistry, 10: 4176-4185, 1971.

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27. Theodore, J., Robin, E.D., Murdaugh, H.V. and Cross, C.E.: Cation transport and energy metabolism in the nucleated erythrocyte of the dogfish shark squalus acanthias. Comp. Biochem. Physiol. 42A: 639-654, 1972.

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30. Robin, E.D., Cross, C.E. and Zelis, R.: Pulmonary edema: Part I, Anatomy, Physiology and Pathophysiology. New Eng. J. Med. 288: 239-246, 1973.

31. Robin, E.D., Cross, C.E. and Zelis, R.: Pulmonary edema: Part II, Clinical Features, functional disturbances and therapy. New Eng. J. Med. 288: 292-304, 1973.

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PUBLICATIONS SUBMITTED

Giri, S.N., Hollinger, M.A., Dungworth, D.L. and Cross, C.E.: Effects of thiourea on pulmonary edema, pleural effusions and peritoneal effusions and toxicity in rats pretreated with actinomycin D.

DeLucia, A.J., Mustafa, M.G. and Cross, C.E.: Ozone interaction with rodent lung. II. Oxidation of reduced glutathione and mixed disulfide formation between proteinand non-protein sulfhydryls.

DeLucia, A.J., Mustafa, M.G. and Cross, C.E.: Ozone interaction with rodent lung. III. Oxidation of reduced glutathione and mixed disulfide formation between protein and non-protein sulfhydryls.

DeLucia, A.J., Mustafa, M.G., Cross, C.E. and Asghar, K.: Increased level of non-protein sulfhydryls in ozone-exposed rodent lungs.

Mustafa, M.G. and Cross, C.E.: Studies of mammalian lung mitochondria: Effects of short-term ozone exposure on oxidative and energy metabolism.

York, G.K., Cross, C.E., Mustafa, M.G., and Bodai, B.I.: Lung glucose-6-phosphate dehydrogenase stimulation by cigarette smoke. Effects of short-term ozone exposure.

Mustafa, M.G., and Cross, C.E.: Effects of short-term ozone exposure on lung mitochomdrial oxidative and energy metabolism.

Mustafa, M.G., and Cross, C.E.: Lung cell mitochondria: Rapid oxidation of glycerol-1-phosphate but slow oxidation of 3-hydroxybutyrate.

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R: REDACTED MATERIAL

CURRICULUM VITAE

Elliot Goldstein, M. D.

REDACTED

Born: REDACTED

Education:

Cornell University - Chemistry, Zoology - REDACTED

Cornell Medical College - REDACTED

Employment:

University of California Hospital - Pathology Internship - July 1960 - June 1961.

Mount Sinai Hospital, New York - Rotating Internship - July 1961 - June 1962.

Bellevue Hospital, First (Columbia) Division - Medical Resident - July 1962 - June 1964.

Boston City Hospital, Channing and Thorndike Fellow in Infectious Disease - Harvard Medical Service - July 1964 - June 1966.

United States Army, Major - Medical Corp, Vietnam - July 1966-67, Fort Devens, July 1967 - 68.

Harvard Medical School - Instructor in Medicine, Director of Tuberculosis Laboratory, Mattapan Sanatorium, Boston City Hospital - July 1968 - July 1969.

University of California at Davis School of Medicine - Assistant Professor of Medicine - August 1969 - June 1971.

University of California at Davis School of Medicine - Associate Professor of Medicine - July 1971 - present.

Society Memberships:

REDACTED

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Professional Activities:

1) Reviewer, Journal of Infectious Diseases
2) Consultant to the Wright-Patterson Research Laboratories, Dayton, Ohio

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PUBLICATIONS
--exclusive of abstracts--

Elliot Goldstein, M. D.

1. Goldstein, E., and Rambo, O. N.: Cryptococcal infection following steroid therapy. Ann. Int. Med. 56:114-120, 1962.

2. Cooper, R. A., and Goldstein, E.: Histoplasmosis of the central nervous system. Am. J. Med. 35:45-57, 1963.

3. Goldstein, E., and Janoski, A. H.: Hemophilus influenzae pyarthroses in an adult. Arch. Int. Med. 114:647-650, 1964.

4. Goldstein, E., Grieco, M. H., Finkel, G., and Louria, D. B.: Studies on the pathogenesis of experimental Candida parapsilosis and Candida guilliermondii infections in mice. J. Inf. Dis. 115:293-302, 1965.

5. Kass, E. H., Green, G. M., and Goldstein, E.: Mechanisms of antibacterial action in the respiration system. Bact. Rev. 30:488-496, 1966.

6. Goldstein, E.: Four important mycotic infections: Histoplasmosis, coccidioidomycosis, cryptococcosis and candidiasis - their present clinical status and significance. Revue de information de corps medicale. 4:377-383, 1966.

7. Goldstein, E., and Green, G. M.: Inhibition of pulmonary bacterial clearance during acute renal failure. Antimicrobial Agents and Chemotherapy--1965. American Society for Microbiology. Ann Arbor, Michigan. 1966. pp. 22-25.

8. Green, G. M., and Goldstein, E.: A method for quantitating intrapulmonary bacterial inactivation in individual animals. J. Lab. Clin. Med. 68:669-677, 1966.

9. Goldstein, E., and Green, G. M.: The effect of acute renal failure on the bacterial clearance mechanisms of the lung. J. Lab. Clin. Med. 68: 531-542, 1966.

10. Goldstein, E., Daly, A. K., and Seamans, C.: Haemophilus influenzae as a cause of adult pneumonia. Ann. Int. Med. 66:35-40, 1967.

11. Goldstein, E., and Green, G. M.: Alteration of the pathogenicity of Pasteurella pneumotropica for the murine lung caused by changes in pulmonary antibacterial activity. J. Bact. 93:1651-1656, 1967.

12. Goldstein, E.: A clinical study of falciparum and vivax malaria in Vietnam servicemen. Military Med. 133:991-996, 1968.

13. Goldstein, E., and Porter, D.: Fatal thrombocytopenia with cerebral hemorrhage in mononucleosis. Arch. Neurol. 20:533-535, 1969.

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15. Fletcher, J., and Goldstein, E.: The effect of parenteral iron preparations on renal infection due to Escherichia coli in rats and mice. Antimicrobial Agents and Chemotherapy--1969. American Society for Microbiology. Ann Arbor, Michigan. 1970. pp. 454-457.

16. Goldstein, E., Green, G. M., and Seamans, C.: The effect of acidosis on pulmonary bactericidal function. J. Lab. Clin. Med. 75:912-923, 1970.

17. Goldstein, E.: Review of the literature pertaining to infection within a spacecraft. In: Infectious Disease in Manned Spaceflight: Probabilities and countermeasures. National Academy of Science. 1970. Appendix A. pp. 102-136.

18. Goldstein, E., and Malloy, M.: Evaluation of the fluorochrome stain in detection of mycobacteria under routine laboratory conditions. J. Conference Pub. Health Laboratory. 28:135-145, 1970.

19. Goldstein, E., Munson, E. S., Eagle, C., Martucci, R. and Hoeprich, P. D.: Influence of anesthetic agents on murine pulmonary bactericidal activity. Antimicrobial Agents and Chemotherapy--1970. American Society for Microbiology. Ann Arbor, Michigan. 1971. pp. 231-235.

20. Fletcher, J. and Goldstein, E.: The effect of parenteral iron preparations on experimental pyelonephritis. Br. J. Exp. Path. 51:280-285, 1970.

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22. Goldstein, E., Tyler, W. S., Hoeprich, P. D., and Eagle, M. C.: The effect of ozone on the anti-bacterial defense mechanisms of the murine lung. Arch. Int. Med. 127:1099-1102, 1971.

23. Goldstein, E., Munson, E. S., Eagle, C., Martucci, R. W. and Hoeprich, P. D.: The effects of anesthetic agents on murine pulmonary bactericidal activity. Anesthesiology. 34:344-352, 1971.

24. Hahn, F. F., Goldstein, E., and Dungworth, D. L.: Effect of whole body x-irradiation on pulmonary bactericidal function. Rad. Research. 47:461-471, 1971.

25. Goldstein, E.: Evaluation of the role of nitrogen dioxide in the development of respiratory diseases in man. California Medicine. 115:21-28, 1971.

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26. Goldstein, E., Eagle, C. and La Casse, M. L.: In vitro chemotherapeutic combinations against isoniazid-resistant *Mycobacterium tuberculosis* and *Mycobacterium fortuitum*. Appl. Micro. 22:329-333, 1971.

27. Goldstein, E., and Hoeprich, P. D.: Problems in the diagnosis and treatment of systemic candidiasis. J. Inf. Dis. 125:190-193, 1972.

28. Goldstein, E., Eagle, M. C., and Hoeprich, P. D.: Influence of ozone on pulmonary defense mechanisms of silicotic mice. Arch. Environ. Hlth. 24:444-448, 1972.

29. Goldstein, E., Buhles, W. C., Akers, T. G., and Vedros, N.: Murine resistance to inhaled *Neisseria meningitidis* after infection with an encephalomyocarditis virus. Inf. & Imm. 6:398-402, 1972.

30. Goldstein, E., Winship, M. J., and Pappagianis, D.: Ventricular fluid and the diagnosis of Coccidioidal meningitis. Ann. Int. Med. 77: 243-246, 1972.

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33. Eisele, John H., Goldstein, Elliot, Martucci, Richard, W., and Eagle, Carroll.: The influence of acute respiratory acidosis on the pulmonary defense mechanisms in rats. Amer. Rev. Resp. Dis. (in press) 1973.

34. Goldstein, Elliot, Eagle, M. Carroll, and Hoeprich, Paul D.: The effect of nitrogen dioxide on the antibacterial defense mechanisms of the murine lung. Arch. Environ. Hlth. 26:202-204, 1973.

35. Goldstein, Elliot, and Lewis, Jerry P.: Patterns of pulmonary alveolar macrophage function following radiation injury. J. Lab. Clin. Med. (in press) 1973.

Chapters in Books

1. Goldstein, E.: Otitis Externa, Chapter 149, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972, Pp. 1267-1271.

2. Goldstein E.: Rat Bite Fever, Chapter 129, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972, Pp. 1149-1152.

3. Goldstein, E., and Hoeprich, P. D.: Diphtheria, Chapter 24, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972, Pp. 269-278.

4. Goldstein, E.: Bartonellosis, Chapter 131, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972, Pp. 1159-1161.

5. Goldstein, E.: Acid-fast staining, in Chapter 8, in Infectious Diseases. Edited by Hoeprich, P. D. Harper & Row, New York, 1972. P. 89.

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R: REDACTED MATERIAL

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CURRICULUM VITAE

NAME Mohammad Ghulam Mustafa

ADDRESS Pulmonary Biochemistry Research Laboratory
California Primate Research Center
University of California
Davis, California 95616

DATE AND PLACE OF BIRTH REDACTED

MARITAL STATUS

NATIONALITY

IMMIGRATION STATUS REDACTED

EDUCATION

Government Muslim High School, Dacca, Bangladesh
Matriculation R

Jagannath College, Dacca, Bangladesh, I.Sc., R

University of Dacca, Dacca, Bangladesh, I.Sc R
M.Sc. in Biochemistry, 1962

University of California, Berkeley, California,
M.A., in Comparative Biochemistry, R

Oregon State University, Corvallis, Oregon, Advanced
to Doctoral Candidacy in Biochemistry. R

State University of New York at Albany. Albany, New
York, Ph.D. in Biochemistry, R

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POSITIONS

1962 - 1963 Teaching and Research Fellow, Department of Biochemistry and Nutrition, University of Dacca, Dacca, Bangladesh

1963 - 1966 Research Assistant, Department of Physiology, University of California, Berkeley, California

1966 - 1968 Research Assistant, Science Research Institute and Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon

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1968 - 1969	Research Assistant, Department of Chemistry, State University of New York, Albany, New York.
1969 - present	Assistant Research Biochemist, Departments of Biological Chemistry and Internal Medicine, School of Medicine, and California Primate Research Center, University of California, Davis, California

SCHOLARSHIPS

1961 - 1962	Departmental Scholarship, University of Dacca, Bangladesh
1963 - 1969	Fulbright Travel Grant, U.S. Educational Foundation in Pakistan (Bangladesh)

GRANTS RECEIVED

Tuberculosis and Respiratory Disease Association of California, "Pulmonary Macrophage: Effects of Common Air Pollutants and Other Noxious Inhalants on Respiration and Energy Production and Utilization Mechanisms". January 1, 1970 to June 30, 1972; $12,600.

Co-investigator in charge of biochemical aspects of lung studies on four other funded multidisciplinary collaborative grants, viz.:

1) National Institute of Environmental Health Sciences, N.I.H., "Pulmonary Effects of Environmental Oxidant Pollutants, June 1, 1971 to June 1, 1975, $780,000 (D. L. Dungworth).

2) American Medical Association Education and Research Foundation Committee for Research on Tobacco and Health "Tobacco Smoke and Lung Biochemistry". February 1, 1971 to February 1, 1973, $60,000 (C. E. Cross).

3) The Council for Tobacco Research - U.S.A., "Effects of Cigarette Smoke on the Pulmonary Lymphoreticular System", January 1, 1970 to January 1, 1973, $120,000 (C. E. Cross).

4) National Institutes of Health, "Health Science Advancement Award in Comparative Medicine", June 1, 1968 to June 1, 1973, Approximately $5,000,000 (R. E. Stowell).

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PUBLICATIONS

1. Mustafa, M.G., Chakraborty, S., Rahman, M.M. and Ahmad, K.: Semimicro determination of uric acid in blood. Pakistan J. Biol. Agri. Sci., 6: 61-69, 1963.

2. Packer, L. and Mustafa, M.G.: Pathways of electron flow established by tetramethylphenylenediamine in mitochondria and ascites tumor cells. Biochem. Biophys. Acta, 113: 1-12, 1966.

3. Mustafa, M.G., Utsumi, K., and Packer, L.: Damped oscillatory control of mitochondrial respiration and volume. Biochem. Biophys. Res. Commun., 24: 381-385, 1966.

4. Packer, L., Utsumi, K., and Mustafa, M.G.: Oscillatory states of mitochondria: I. Electron and energy transfer pathways. Arch. Biochem. Biophys. 177: 381-393, 1966.

5. Mustafa, M.G. and King, T.E.: Wurster's blue mediated oxidation of NADH and phosphorylation in mitochondria. Arch. Biochem. Biophys., 122: 501-508, 1967.

6. Mustafa, M.G., Cowger, M.L. and King, T.E.: On the energy-dependent bilirubin-induced mitochondrial swelling. Biochem. Biophys. Res. Commun., 29: 661-666, 1967.

7. Mustafa, M.G., Cowger, M.L., Labbe, R.F. and King, T.E.: General nature of "Wurster's blue shunts" in the respiratory chain. J. Biol. Chem., 243: 1908-1918, 1968.

8. Mustafa, M.G., Cowger, M.L. and King, T.E.: Effects of bilirubin on mitochondrial reactions. J. Biol. Chem., 244: 6403-6414, 1969.

9. Mustafa, M.G., Ibrahim, A.B., Le, C.T. and Cross, C.E.: Pulmonary alveolar macrophage: Demonstration of Na^{+}-K^{+}, Mg^{++} ATPase activity. Life Sci., 8: 1343-1351, 1969.

10. Mustafa, M.G. and King, T.E.: Binding of bilirubin with lipid: a possible mechanism of its toxic reaction in mitochondria. J. Biol. Chem., 245: 1084-89, 1970.

11. Mustafa, M.G., and Cross, C.E.: Localization of Na^{+}-K^{+}, Mg^{++} ATPase in alveolar macrophage subcellular fractions. Life Sci., 9: 947-54, 1970.

12. Cross, C.E., Ibrahim, A.B., Ahmed, M., and Mustafa, M.G.: Effects of Cd^{++} on respiration and ATPase activity of the pulmonary alveolar macrophage: A model for the study of environmental interference with pulmonary cell function. Environ. Res., 3: 512-520, 1970.

13. Meyer, D.H., Cross, C.E., Ibrahim, A.B. and Mustafa, M.G.: Nicotine effects on alveolar macrophage respiration and adenosine triphosphatase activity. Arch. Environ. Health, 22: 362-365, 1971.

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14. Mustafa, M.G., Cross, C.E., and Tyler, W.S.: Interference of cadmium ion with oxidative metabolism of alveolar macrophages. Arch. Int. Med., 127: 1050-1058, 1971.

15. Cross, C.E., Mustafa, M.G., Peterson, P., and Hardie, J.A.: Pulmonary alveolar macrophage: Membrane associated Na^{+}, K^{+}, Mg^{++}-adenosine triphosphatase system. Arch. Int. Med., 127: 1069-1077, 1971.

16. Mustafa, M.G., Peterson, P.A., Munn, R.J., and Cross, C.E.: Effects of Cd^{++} on metabolism of lung cells. In Proceedings of the Second International Clean Air Congress, Edited by H.M. Englund and W.T. Berry, Academic Press, N.Y., p. 143-151, 1971.

17. Mustafa, M.G., and Cross, C.E.: The pulmonary alveolar macrophage: Oxidative metabolism of isolated cells and mitochondria and effect of cadmium ion on electron and energy transfer reactions. Biochemistry, 19: 4176-4185, 1971.

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19. Utsumi, K., Torres-Pereirae, Mustafa, M.G., and Oda, T.: Change of proton gradient in mitochondria at various energy states. Acta Med. Okayama, 25: 493-504, 1971.

20. Mustafa, M.G., Le, C.T., Harris, N.E., and Cross, C.E.: Effects of proteases and lipases on membrane associated biochemical reactions of alveolar macrophage and lung tissue. In Pulmonary Emphysema and Proteolysis, C. Mittman, Editor, Academic Press, N.Y., p. 281-294, 1972.

21. DeLucia, A.J., Hoque, P.M., Mustafa, M.G., and Cross, C.E.: Ozone interaction with rodent lung: Effect on sulfhydryls and sulfhydryl-containing enzyme activities. J. Lab. Clin. Med., 80: 559-566, 1972.

22. York, G.K., Arth, C., Stumbo, J.A., Cross, C.E. and Mustafa, M.G.: Effect of cigarette smoke and tobacco extract on lung macrophage respiration. Arch. Environ. Health, in press, 1973.

23. Mustafa, M.G., DeLucia, A.J., York, G.K., Arth, C. and Cross, C.E.: Ozone interaction with rodent lung. II. Effects on oxygen consumption of mitochondria. J. Lab. Clin. Med., in press, 1973.

24. Mustafa, M.G., DeLucia, A.J. and Cross, C.E.: Effect of ozone exposure on lung mitochondrial oxidative metabolism. Chest, in press, 1973.

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Manuscripts submitted or in preparation (1973)

1. DeLucia, A.J., Mustafa, M.G. and Cross, C.E.: Ozone interaction with rodent lung. III. Oxidation of reduced glutathione and mixed disulfide formation between protein and nonprotein sulfhydryls.

2. DeLucia, A.J., Mustafa, M.G., Cross, C.E. and Asghar, K.: Increased level of nonprotein sulfhydryls in ozone-exposed rodent lungs.

3. Mustafa, M.G. and Cross, C.E.: Studies of mammalian lung mitochondria: Effects of short-term ozone exposure on oxidative and energy metabolism.

4. Mustafa, M.G., DeLucia, A.J., Cross, C.E., Dungworth, D.L. and Tyler, W.S.: Biochemical and morphological alterations in the lung following ozone exposure.

5. Cytoplasmic and microsomal membrane fractions of lung: Adenosine triphosphatase activity.

6. Lung monoamine oxidase. I. Substrate utilization and subcellular localization. II. Effect of acute and chronic ozone exposure.

7. Lung mitochondria: Increased oxidation of glycerol-1-phosphate in chronic ozone exposure.

8. Increased mitochondrial enzymatic activities in the lung during recovery from acute ozone exposure.

9. Increased enzymatic activities for sulfhydryl metabolism activities in the lung in chronic ozone exposure.

10. Biological effects of atmospheric pollutants, a chapter for the book on "Atmospheric Sciences".

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ABSTRACTS

1. Mustafa, M.G. and Packer, L.: Interaction of tetramethylphenylenediamine with rat liver mitochondria. Pacific Slope Biochemical Conference (Presented at August meetings at Eugene, Oregon, 1966).

2. Mustafa, M.G. and King, T.E.: Wurster's blue mediated oxidation of NADH and associated phosphorylation in mitochondria. Pacific Slope Biochemical Conference (Presented at June meetings at Davis, California, 1967).

3. Mustafa, M.G.: General nature of the interactions of TMPDox with the mitochondrial respiratory chain. Pacific Slope Biochemical Conference (Presented at June meetings at Davis, California, 1967).

4. Mustafa, M.G. Cowger, M.L. and King, T.E.: Energy-dependent bilirubin-induced swelling. Fed. Proc., 27, 833, 1968.

5. Mustafa, M.G.: Mechanism of bilirubin action affecting processes manifested by mitochondrial membrane. Fed. Proc., 28, 822, 1969.

6. Mustafa, M.G., Ibrahim, A.B., Tyler, W.S. and Cross, C.E.: Pulmonary alveolar macrophage: Demonstration of Na^{+}-K^{+}, Mg^{++} ATPase activity. Fed. Proc., 29, 663, 1970. (Presented at FASEB American Physiologic Society, April 12-17 meetings at Atlantic City).

7. Mustafa, M.G., Ibrahim, A.B., Hance, A.J. and Cross, C.E.: Pulmonary alveolar macrophage: Inhibition of electron and energy transfer by cadmium ion in whole cells and mitochondria. Fed. Proc., 29, 911, 1970.

8. Cross, C.E., Ibrahim, A.B., and Mustafa, M.G.: Pulmonary alveolar macrophage: Effects of divalent cations on respiratory and energy transfer systems. Clin. Res., 18, 483, 1970.

9. Mustafa, M.G., T.S. Groves and Cross, C.E.: Contraction and aggregation phenomena in pulmonary alveolar macrophages (PAMs). Clin. Res., 19, 138, 1971.

10. Cross, C.E., Le, C.T. and Mustafa, M.G.: Effects of ozone on some pulmonary alveolar macrophage (PAM) biochemical reactions. Clin. Res., 19, 147, 1971.

11. Mustafa, M.G. and Cross, C.E.: Oxidative metabolism of pulmonary alveolar macrophages. Eighth National Scientific Meeting of the Reticuloendothelial Society, 1971. (Presented at November 30-December 3 meetings at Detroit).

12. Mustafa, M.G., Cronin, S.R., Duvall, T.R. and Cross, C.E.: Ozone effect on activities of lung cellular membrane enzymes. Clin. Res. 20: 197, 1972.

13. DeLucia, A.J., Hoque, P.M., Cross, C.E. and Mustafa, M.G.: Sulfhydryl content of rodent lungs under oxidant stress. Clin. Res. 20: 240, 1972.

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14. Mustafa, M.G., Belleci, P. and Cross, C.E.: Oxidation of biological amines by mammalian lung tissue. Fifth International Congress of Pharmacology, 1972 (Presented at July 23-28 meetings at San Francisco).

15. Mustafa, M.G. and Cross, C.E.: Effect of tobacco smoke on lung cellular membrane enzymes. Comittee for Research on Tobacco and Health, American Medical Association Education and Research Foundation, Third Research Conference, 1972. (Presented at May 7-9 meetings at Newport Beach, Calif.).

16. DeLucia, A.J., Mustafa, M.G. and Cross, C.E.: Glutathione and sulfhydryl-containing enzyme activities in lung under ozone stress. Clin. Res. 21, 223, 1973.

17. Mustafa, M.G., Cross, C.E. and Tyler, W.S.: Ozone wxposure causing biochemical and anatomical alterations in the lung. Fed. Proc. 32, 864, 1973. (Presented at FASEB American Pathological Society, April 15-20 meetings at Atlantic City).

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DISSERTATIONS

1. Mustafa, M.G.: On an antiuricemic principle from Boerhaavia diffusa linn -- a dissertation for Master of Science in Biochemistry submitted to the University of Dacca, Dacca, Bangladesh (1962).

2. Mustafa, M.G.: Oxidation of tetramethylphenylenediamine by mitochondria -- a dissertation for Master of Arts in Comparative Biochemistry submitted to the University of California, Berkeley, California (1966).

3. Mustafa, M.G.: Certain electron and energy transfer reactions of mitochondria -- a dissertation for Doctor of Philosophy in Biochemistry submitted to the State University of New York at Albany, Albany, New York (1969).

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R: REDACTED MATERIAL

Ching-Kuang Chow
Department of Food Science & Technology
University of California
Davis, California 95616
Telephone: 916-752-3684

home

REDACTED

REDACTED

PERSONAL

EDUCATION

National Taiwan University, Taipei, Taiwan, REDACTED in Ag. Chemistry.
Univ. of Illinois, Urbana, Illinois, in Animal Nutrition.
Univ. of Illinois, Urbana, Illinois, REDACTED in Nutritional Sciences, minors in Biochemistry and Food Science.

RESEARCH AND PROFESSIONAL EXPERIENCE

1972-present, Assist. Research Biochemist, Univ. of Calif., Davis, Calif. Oxidant induced lipid peroxidation damage; antioxidant function of SH- compounds, Se and vitamin E; relationship between Se and glutathione peroxidase; mechanism of lipid peroxidation associated enzyme alteration; automation of enzyme assay; enzyme purification; lipid peroxidation related aging (fluorescent) pigments and pathogenic changes.

1971-1972, National Vitamin Foundation Fellow, Univ. of Calif., Davis, Calif. Air pollution oxidant damage and antioxidant protection; enzymatic protection against lipid peroxidation damage; tissue enzyme distribution; enzymes of carbonhydrate metabolism; alteration of enzymic activity as a sensitive indicator for tissue damage; lipid peroxide preparation and detoxification mechanism.

1970-1971, Research Associate, Univ. of Illinois, Urbana, Illinois Turn-over rates of tocochromanols; toxicity of NO_2 and nitrate (and nitrite); Mississippi Holmes County Nutrition Survey; phospholipids, sterols and carotenoid compounds on the stability of oils.

1969-1970, Assoc. Research Scientist, New York Univ. Medical Center, N. Y. Assay, absorption, transportation and metabolism of α-tocopherol in human blood; lipid composition of erythrocyte membranes.

1964-1969, Graduate Research Assistant, Univ. of Illinois, Urbana, Illinois Chemistry and biochemistry of tocopherols and related compounds; synthesis of radioactive tocopherols and tocopheryl quinone; methods for lipid and tocopherol assay; isolation and identification of organic compounds; oxidative stability of tocopherols fatty acids and oils; preparation of hydroperoxide and hydroxy fatty acids, and metabolism; vitamin A deficiency and oxidative phosphorylation; lipid biosynthesis in root tissue.

1963-1964, Researcher, Subsistence Factory, CSF, Taichung, Taiwan Inspection, research and development of canned foods and combat rations.

1962-1963, Senior thesis research, National Taiwan Univ., Taipei, Taiwan Protein and amino acid nutrition.

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HONORS

Research assistantship, Univ. of Illinois, 1964-1969; American Society for Clinical Nutrition Research Fellowship, 1971-1972; Sigma Xi Grant-in-aid for Research Award, 1972; listed in American Men & Women of Science. Members of Sigma Xi and New York Academy of Sciences.

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List of Publications

1. Chow, C. K. Effect of d,l-methionine supplementation on growth, nitrogen retention and energy metabolism of the growing rats. B.S. thesis, National Taiwan University, 1963.

2. Chow, C. K. Metabolism of α-tocopheryl quinone and α-tocopheryl hydroquinone. M.S. thesis, University of Illinois, 1966.

3. Chow, C. K. Studies on the assay, stability, antioxidant activity and oxidation products of tocopherols. Ph.D. thesis, University of Illinois, 1969. Diss. Abstr. Int. B. 31, 559, 1970.

4. Chow, C. K., H. H. Draper, A. S. Csallany and M. Chiu. The metabolism of C-14-α-tocopheryl quinone and C-14-α-tocopheryl hydroquinone. Lipids 2, 390, 1967.

5. Chow, C. K., H. H. Draper and A. S. Csallany. Method for the assay of free and esterified tocopherols. Anal. Biochem. 32, 81, 1969.

6. Chow, C. K. and H. H. Draper. Effect of artificial drying on the tocopherols and fatty acids of corn. J. Agri. Food Chem. 17, 1316, 1969.

7. Chow, C. K. and H. H. Draper. Isolation of γ-tocotrienol dimers from Hevea latex. Biochemistry 9, 445, 1970.

8. Chow, C. K. and H. J. Kayden. Tocopherol levels in human erythrocytes. Fed. Proc. 29, 693, 1970abs.

9. Chow, C. K., A. S. Csallany and H. H. Draper. Turnover rates of tocochromanols in rabbit plasma. Nutr. Report Int'l. 4, 45, 1971.

10. Csallany, A. S., C. K. Chow and H. H. Draper. Relationship between erythrocyte and plasma vitamin E in the rabbit. Nutr. Report Int'l. 4, 325, 1971.

11. Fletcher, B. L., K. Reddy, C. J. Dillard, C. K. Chow and A. L. Tappel. Fluorescence measurement of lipid peroxidation and the effect of dietary vitamin E on biochemical damage in rats exposed to O_3 and NO_2. Fed. Proc. 31, 713, 1972abs.

12. Chow, C. K. and A. L. Tappel. An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. Lipids 7, 518, 1972.

13. Chow, C. K. and A. L. Tappel. Glutathione peroxidase system protects against oxidative damage in lungs of ozone-exposed rats. Clin. Res. 21, 277, 1973abs.

14. Chow, C. K. and A. L. Tappel. Activities of pentose shunt and glycolytic enzymes in lungs of ozone-exposed rats. Arch. Environ. Health, 26, 618, 1973.

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List of Publications (continued)

15. Chow, C. K., K. Reddy and A. L. Tappel. Effect of dietary vitamin E on the activities of the glutathione peroxidase system in rat tissues. J. Nutr. 103, 618, 1973.

16. Chow, C. K. On the nitrates, nitrites and nitrosamines in foods. Food Industries (Taiwan), in press, 1973.

17. Kayden, H. J. and C. K. Chow. Spectrophotometric method for the determination of α-tocopherol in red blood cells. ~~Submitted to~~ J. Lipid Research, IN Press, 1973.

18. Chow, C. K., C. J. Dillard and A. L. Tappel. Glutathione peroxidase system and lysozyme in rats exposed to ozone and nitrogen dioxide. Submitted to Environ. Res.

19. Smith, P, A. L. Tappel and C. K. Chow. Glutathione peroxidase activity as a function of dietary selenomethionine. Submitted to Science.

20. Chow, C. K. On the existence of lipid peroxides in vivo and biological function of vitamin E. Submitted to J. Chinese Agri. Chem. Soc.

21. Chow, C. K. and A. L. Tappel. Kinetic and mechanism of glutathione peroxidase activity in rat tissues as influenced by dietary selenomethionine. In preparation for J. Nutr.

22. Chow, C. K. and A. L. Tappel. Normal glutathione peroxidase activity in Batten's syndrome. In preparation for Nature.

23. Chow, C. K. and H. H. Draper. Oxidative stability of tocopherols in vegetable oils. In preparation for J. Am. Oil Chemists' Soc.

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